Environmental Health Perspectives
Vol 90, pp. 165-169, 1991

Advances in Early Fetal Loss Research: Importance for Risk Assessment

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The assessment of early fetal losses (EFLs) in relationship to environmental agents offers unique advantages compared to other end points for hazard assessment. There is a high incidence (> 20% of all pregnancies end in an EFL), and the interval between exposure and end point is the short duration between conception and event, i.e., approximately 12 weeks. In contrast, cancer, which is the primary end point evaluated in risk assessment models, occurs with much lower frequency, and the latency period is measured in years or decades. EFLs have not been used effectively for risk assessment because most of the events are not detected. Prospective studies provide the only approach whereby it is possible to link exposure to EFLs. Recent methodologic advancements have demonstrated that it is now possible to conduct population-based studies of EFLs. It is likely that EFLs could serve as sentinels to monitor adverse health effects of many potential environmental hazards. The methodology will be demonstrated using lead exposure *in utero* as an example.

Introduction

During the last 5 years, considerable advances have been made in the investigation of the relationship of environmental exposures to early fetal losses (EFLs). Given these developments, it is possible that EFLs may become an optimum end point that could be used to formulate standards and policies concerning the contribution of environmental factors to adverse human health effects. As Wilson and Crouch (*1*) stated so succinctly in 1987: "There have been few attempts to perform risk assessment for biological ends point other than cancer." Perhaps with the use of EFLs as an end point, more accurate risk assessments of health outcomes will be performed.

Advantages of Assessing Early Fetal Losses

It is important to put the early fetal loss research in the context of established approaches for risk assessment. Russell and Gruber (*2*) reviewed the risk assessment procedures at the Environmental Protection Agency. The steps included hazard assessment; dose-response assessment; exposure assessment; and risk characterization. The cornerstone of the approach is hazard assessment, as without hazard assessment the other steps are irrelevant. However, epidemiologic methods and technology are much cruder than the more sophisticated toxicological, biostatistical, and exposure measurements that have been developed by colleagues in other fields. Despite the difficulty of hazard assessment, the epidemiologic work linking environmental agents to acute and chronic health problems in humans the most important data for risk assessment. We argue that the addition of early fetal loss studies to our repertoire of hazard assessment could complement the risk assessment that has been developed through the examination of cancer, thus considerably improving our knowledge of the health effects of factors in the environment.

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What Are the Advantages of Assessing Early Fetal Losses for Hazard Assessment?

Advantage 1: Incidence

A central difficulty in using cancer as the basis for risk assessment has been the low frequency of occurrence. To evaluate the relationship of environmental factors to cancer requires large sample sizes and enormous numbers of person years of follow-up to provide sufficient power to detect associations. For many of the environmental exposures, such as toxic waste dumps, a limited number of people are exposed for short periods of time. Therefore, studies of such exposures are likely to be negative solely due to insufficient power to detect an association between the environmental factor and cancer.

The incidence of cancer per year on a population basis is only 2.8 per 1000, most of which occurs in older individuals. When only one person in 350 per year develops cancer, it is obviously difficult, if not impossible, to do meaningful prospective studies. Moreover, most of the focus of environmental work has been with site-specific cancers where a much lower incidence results in the need for even larger samples. When examining site-specific cancers, the sample sizes are so large that it often precludes even a retrospective study.

In contrast, EFLs have a much higher incidence: more than 20% of all pregnancies result in an early pregnancy loss. Human reproduction has been described as an "inefficient" process (*3*).

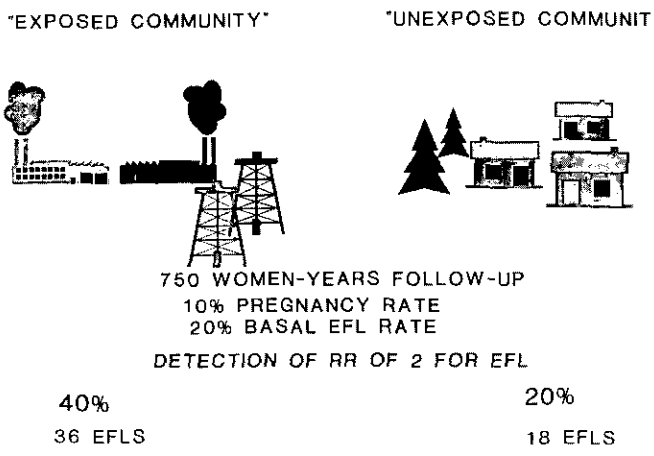

FIGURE 1. Number of end points needed to detect a 2-fold increase in EFLs in exposed compared to unexposed communities. These calculations are based on a pregnancy rate of 10% and an EFL rate of 20% in the control population.

The pregnancy rate per year for women of childbearing age in the U.S. is 10%. Overall, the annual incidence of early fetal losses for women of childbearing age therefore is 0.20×0.10 or about 2%, which is almost 10 times that of cancer. If the population were restricted to the 50% of women who are "susceptible" to becoming pregnant (i.e., those not sterilized, or on effective means of contraception), the incidence would be 4% or almost 20 times that of cancer. Restricting this even further to women who are at risk of early fetal loss (i.e., those who become pregnant), the incidence of early fetal loss is 100 times greater than that of cancer.

To illustrate this further, an example is presented in relationship to lead exposure. High-level lead exposure has been documented to be an abortifacient (*4*), but we know little about the effect of low levels of exposure. Figure 1 presents two hypothetical communities, one exposed to a lead smelter, and the other unexposed. Suppose that a study is being designed to detect a 2-fold difference in the risk of early fetal losses in each of these two communities. Assuming a basal EFL rate of 20%, the goal is to have sufficient power to detect a 2-fold, or 40% incidence of EFL in the exposed community. Based on a 10% pregnancy risk for women of childbearing age, the sample size ($\alpha = 0.05$, $\beta = 0.80$, one-tailed test) required would consist of 750 women from each community followed for 1 year, or 375 women for 2 years, or 250 women for 3 years (*5*). With sample sizes such as this, prospective studies are indeed feasible.

Advantage 2: Latency

Carcinogenic risk estimation is complicated by the time delay between onset and detection of the cancer, which is one reason why causality is difficult to prove (*1*). Clearly, the latency between exposure and cancer outcome injects considerable uncertainty in determining causality, or even in discovering any association except those of the strongest magnitude. As has been discussed by numerous authors, recalling or reconstructing exposure over extended time periods is very uncertain. The latency period between exposure and cancer is typically measured in decades, often resulting in unreliable measures of exposure.

In contrast, the interval between exposure and outcome for early fetal loss is not decades or years but rather weeks or even days. This vastly improves our ability to obtain a much more precise estimate of the exposure at the time in which the environmental insult produces the adverse outcome. The first 12 weeks of pregnancy, i.e., the period of organogenesis, is the time of most rapid cell growth and division in humans, and thus a time when the organism is most vulnerable. The fetus is therefore at greatest risk from environmental insults during a very short 12-week time period. Data collection during these 12 weeks results in exposure documentation at the time in which the insult can produce the EFL. Thus, much greater precision is available to link environmental factors to early fetal losses than to link environmental factors to most cancers.

Given the advantages of assessing early fetal losses, why has this end point not been used to a much greater extent in risk assessment work? Until the past few years, examination of early fetal losses has been viewed as a daunting task. Few investigators thought it was feasible to capture a large percentage of pregnancies, and thus early fetal losses, on a population basis. It is important to review the epidemiology of EFLs, as this directly relates to the difficulty of conducting research.

As presented in Figure 2, the vast majority of early fetal losses occur early in pregnancy. The vertical line illustrates the typical time at which pregnancies are diagnosed on a population basis. By 9 to 10 weeks gestation, over 60% of the events have occurred (*6*). Should a woman suffer an early fetal loss prior to this time, she likely would not have known of the pregnancy and would have attributed the loss to a late, heavy menstrual flow. Therefore, the only method by which it is possible to identify a large percentage of end points is through a prospective study in which there is an early diagnosis of pregnancy and the women are then followed to either an early fetal loss or a term pregnancy.

The methodology for diagnosing pregnancy has changed dramatically in the last 10 years. At approximately 7 days postconception, there is a rapid rise in human chorionic gonadotropin hormone (hCG) in both serum and urine. In the early 1980s, the most sensitive tests available for measuring hCG were those performed on sera. It would be extremely difficult, however, to systematically collect sera from a population-based sample. However, in the past few years, urine tests with sensitivity equal to that of serum assays have become available. Use of these urine tests has resulted in rates of early fetal loss ranging from 18 to 31% (*7,8*).

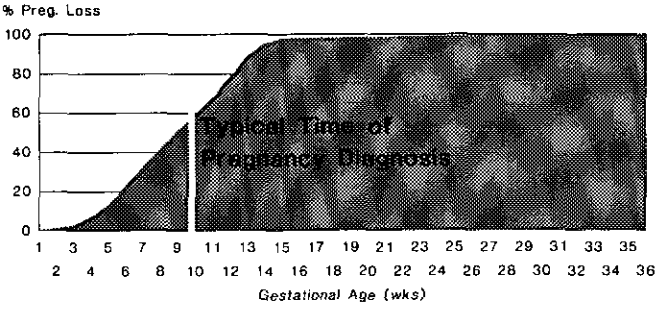

FIGURE 2. Rates of early fetal loss according week of gestation.

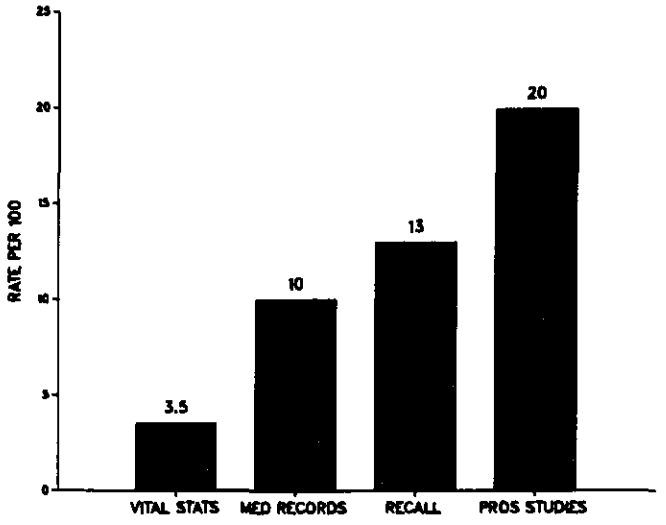

FIGURE 3. Rates of fetal loss according to method of ascertainment.

A major difficulty of environmental work in the area of early fetal losses has been the reliance on either vital records or recall. Figure 3 presents the incidence of early fetal loss based upon the various methods of ascertainment. With vital records, hospital records, and recall, the vast majority of early fetal losses are not detected. To cite a more specific example, there is considerable controversy as to the relationship (if any) between pesticide use and early fetal losses. Figure 4 presents several studies that have been completed, all of which have employed either vital records or recall. As shown, little association has been found between pesticide exposure and early fetal losses. However, as illustrated, the vast majority of the events were not detected in both the exposed and the unexposed groups. Clearly, it would be almost impossible to find an association given that most of the events are missed. The existing studies identify only the tip of the iceberg of fetal losses.

The second and perhaps more formidable task is that of recruitment. Researchers have tended to shy away from prospective population studies of early fetal losses because of the view that recruitment was too formidable, as it would be necessary to recruit not only women planning to become pregnant but also those who were not. On a population basis, only 50% of all pregnancies are planned (*9*). Therefore, by following only volunteers attempting to conceive, one-half of the pregnancies

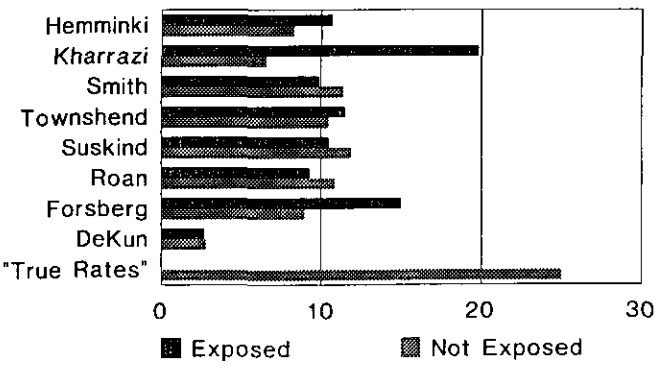

FIGURE 4. Early fetal loss in retrospective studies of pesticide exposure.

would not be evaluated. In addition, women who plan their pregnancies are quite different from those who do not with regard to lifestyle factors such as smoking, drug use, and alcohol consumption (*10*). They are also from higher socioeconomic classes, which can be a confounder in studies of adverse health outcomes. It is likely that exposure would be greater among women who conceive without having planned the pregnancy.

Prospective pregnancy studies have attempted to recruit women by three different methods. Wilcox and colleagues have followed volunteer women who were attempting to become pregnant and have demonstrated excellent compliance (*8*). Ellish et al. have been successfully recruiting a population based cohort of women who are planning to become pregnant (*11*). Recruitment appears to be very good with this group also. We have taken on the difficult task of trying to recruit women at risk of pregnancy into a study, regardless of desire to become pregnant (*12*). Fifty-two percent of eligible women approached in a low socioeconomic community participated; only 2% of whom were planning pregnancy. For the original group of randomly selected women, 3.5 pregnancies would have been expected; four were identified. Two occurred in women planning pregnancy and two were unplanned pregnancies. Thus, with only 52% of the population recruited, a large percentage of the pregnancies occurring in the population were being ascertained. It appeared that women recruited by this approach were those in whom the majority of pregnancies occurred. Thus, it appears that the technology and recruitment methods potentially are available to identify a large percentage of the EFLs that are occurring on a population basis. How might these approaches be used?

Risk Assessment with Lead

The current recommendation for blood lead levels is less than 25 μg/dL (*13*). Many investigators believe that this is insufficient and that the standards should become more stringent, i.e., < 15 μg/dL. To achieve these standards would require millions and perhaps billions of dollars, as it would potentially involve remedial work in much of the housing built in the United States during the early part of the century, which often contains lead-based paint.

The major reason for concern is that lead appears to be a neurotoxin both in animals and humans. Researchers have indicated that lead exposure early in life in humans may result in intellectual deficits later in life (*14,15*). As we have reviewed (*16*), there also is evidence for transplacental lead absorption in animals. Moreover, lead at high levels appears to be both an abortifacient (*4*) and a teratogen (*17*), thus suggesting that the point at which humans are most vulnerable is during organogenesis.

There are no blood lead standards for women of childbearing age; however, this may be at least as important as the standards established for children. To set lower standards, such as 15 μg/dL, would be extremely costly. Clearly there is a need for risk assessment, however, very few human data are available for developing even a reasonable hazard assessment model.

To evaluate reproductive hazards associated with low to moderate lead exposure would be quite simple. Many potentially exposed populations have been identified through the Centers for Disease Control-sponsored lead screening programs, primarily in urban areas with older homes. A prospective population-

based surveillance of women of childbearing age could be established. Women would be identified as being pregnant no later than the third week of gestation, employing urine assays for hCG. Once pregnancy is diagnosed (within a day after obtaining the urine sample) a blood lead determination could be obtained. Because the half-life of lead in blood is approximately 14 days, this determination would reflect the lead concentration at or near the time in which the early fetal loss may occur. With this design, women having an early fetal loss could be compared with those who did not. One could then easily compute the significance and odds ratio for varied lead concentrations. This approach would enable one to develop an accurate risk assessment model.

Obviously, a similar approach could be used with pesticides, occupational exposures such as organic compounds, or exposures near a toxic waste dump. The unique advantage of this method is that exposures can be measured during the short window of susceptibility where the environmental events producing the EFL are occurring. Currently, therefore, the prospective assessment of early fetal losses holds bright promise for risk assessment.

Host Susceptibility and Risk Assessment

One might speculate as to the future of risk assessment, especially with regard to early fetal losses. A fundamental difficulty of hazard assessment is that despite large numbers of people being exposed, only a few are susceptible to developing the disease. Using the most extreme example, as indicated in a review of risk assessment by Khoury et al., only 13% of smokers are susceptible to lung cancer from smoking (*18*). Disease is a product of environmental exposure and host susceptibility; however, the host susceptibility component has not been incorporated into hazard assessment. It is likely that hazard assessment would dramatically improve, and our concept of risk assessment would change, if we were able to define more precisely who is and who is not at risk of disease when exposed to environmental events. To cite an example, if a pesticide were associated with a 10-fold increased risk of EFLs but only 5% of the study population was susceptible, the overall relative risk would be considerably below 2, thus masking a true association in the highly vulnerable subgroup in the population.

The study of early fetal losses offers a unique advantage for incorporating host susceptibility into environmental epidemiology studies and could serve as a model for several other outcomes. There is evidence that the risk of early fetal loss is dependent upon certain genes in the major histocompatibility locus on chromosome six (*19*). These genes are called HLA (human lymphocyte antigen). The HLA region represents the major genetic area defining immunologic response and is one of the most polymorphic regions of the human genome. HLA antigens are present in lymphocytes and play a role in immune surveillance in identifying foreign antigens. Individuals can be categorized by the specific HLA antigens that they inherit from their parents.

Pregnancy is a unique condition in that in order for a pregnancy to be successful (i.e., reach term), it appears the mother's immune system must recognize the conceptus as a foreign presence, thus eliciting an appropriate maternal response. Extensive examination of the HLA system in the area of transplantation has shown that HLA antigens are responsible for the recognition of foreign tissue. The more similar two individuals are with regard to the HLA region, the lower the likelihood of rejection. However, in pregnancy it appears that the failure to recognize this foreign tissue (conceptus) may result in an early fetal loss. This process appears to be at least partially under the control of the HLA region. Thus host susceptibility for EFLs may potentially be defined by HLA markers. Incorporation of these markers into environmental studies of EFLs could be used to assess directly the interaction between host and environment that results in the adverse outcome.

Traditionally, epidemiologic investigations have permitted us to establish the population relative risk or the population attributable risks; however, these measures are not very accurate as the vast majority of the population is not at risk for health hazards due to most environmental agents. The incorporation of new genetic technology to identify susceptible individuals will permit risk assessment for specific chemicals exclusively among those who are at risk of the adverse outcome.

Conclusion

In reviewing the risk assessment literature, it is clear that the fundamental building block is hazard assessment in humans. Hazard assessment, however, has typically been the weakest link of the four steps in the risk assessment process. We believe that human hazard assessment can be strengthened considerably if, in addition to cancer, we begin to employ early fetal loss as an end point.

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